Synthesized ectomycorrhizae of aspen: fungal genus level of structural characterization

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The ability of *Populus tremuloides* Michx. to form ectomycorrhizae with identified species of fungi was investigated using a pouch technique. Twenty-nine out of 54 fungus species formed ectomycorrhizae on aspen seedlings. Aspen seems to display little specificity for ectomycorrhizal fungi. Only epidermal Hartig nets were observed in the synthesized ectomycorrhizae and periepidermal ones were frequently encountered. Structural and morphological characteristics of ectomycorrhizae are presented by genus of mycorrhizal fungi. This grouping of characters by genus seems possible independently of the host plant. The classification of ectomycorrhizae is viewed in a new way in which structural types would be taxonomically related to the genus of the fungal symbiont.

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La capacité de *Populus tremuloides* Michx. à former des ectomycorhizes avec des espèces déterminées de champignons a été explorée au moyen de la technique du sachet de croissance. Sur 54 espèces de champignons essayés, 29 ont formé des ectomycorhizes sur des plantules. Le peuplier faux-tremble semble afficher peu de spécificité vis-à-vis les champignons ectomycorhiziens. Chez les ectomycorhizes obtenues, le réseau de Hartig a toujours été épidermique et de nombreux réseaux périépidermiques furent observés. Les caractéristiques structurales et morphologiques des ectomycorhizes ont été regroupées et décrites par genre de champignons ectomycorhiziens. Cette façon de regrouper les caractères semble possible sans tenir compte de la plante-hôte. La classification des ectomycorhizes est envigagée dans une nouvelle optique selon laquelle les types structuraux seraient taxonomiquement reliés au genre du champignon symbiotique.

Introduction

The genus *Populus* has a widespread geographic distribution, and many species possess a high growth potential. Poplars are able to grow on poor or disturbed soils and are usually considered to be early colonizers. Recently they have been used in the reclamation of spoil banks (Harris and Jurgensen 1977; Shuffstall and Medve 1979) and in biomass plantations using hybrid poplars (De Bell and Radwan 1979).

Populus species can form both ectomycorrhizae and endomycorrhizae, but most roots are ectomycorrhizal in the field. Species that form endomycorrhizae under all conditions are unknown (Dominik 1958; Vozzo and Hacskaylo 1964; Schramm 1966). Endomycorrhizae predominate on *P. balsamifera* L., *P. deltoides* Bartr., and *P. nigra* L. (Dominik 1958; Vozzo and Hacskaylo 1964; Malloch and Malloch 1982).

A wide variety of ectomycorrhiza types occur on poplars (Dominik 1958; Mejstrik 1971), but specific ectomycorrhizal fungus associates are not as well known. From field observations, five fungus species have been found ectomycorrhizal with poplars: *Cenococcum geophilum* Fr. (Trappe 1962), *Hebeloma hiemale* Bres. (Fontana 1963), *H. longicaudum* (Fr. ex Pers.) Quél. (Fontana 1961), *T. borchii* Vitt. (Mattirolo 1934), and *T. magnatum* Pico. (Mattirolo 1935). In pure culture, Melin (1923) synthesized ectomycorrhizae on *Populus tremula* L. with *Leccinum scabrum* (Fr.) S. F. Gray and *L. aurantiacum* (Bull.: St-Amans) S. F. Gray and Fontana and Palenzona (1969) with *Tuber albidum* on hybrid poplars in pots.

The aims of this report are (*i*) to evaluate the ability of *Populus tremuloides* Michx. to form ectomycorrhizae with identified potentially ectomycorrhizal fungi and (*ii*) to describe the structure and morphology of the synthesized ectomycorrhizae along with their extramatrical phase.

Materials and methods

Fungal isolates

Table 1 lists the fungal isolates used. All were isolated from sporophore tissues except for the ascomycetes. *Gyromitra esculenta* and *Morchella conica* cultures were obtained from germinated ascospores, and *Cenococcum geophilum* isolates XX-50 and DGL 80221E.15 were grown from surface-sterilized ectomycorrhiza of *Picea mariana* (Mill.) B.S.P. and sclerotium, respectively. MMN agar medium (Marx and Bryan 1975) was used to maintain the cultures. Fungi were isolated by the first author (C.G.) unless otherwise noted.

Growth-pouch method

Previously frozen seeds of *Populus tremuloides* were germinated on 70-mesh silica sand and moistened with H_2O in Petri dishes. Two-day-old seedlings were transplanted into sterile 70-mesh silica sand in open containers under nonaseptic conditions and saturated with the following autoclaved, mineral solution: $(NH_4)_2HPO_4$, 0.26 g; NH_4NO_3, 0.16 g; KCl, 0.52 g; $CaCl_2 \cdot 2H_2O$, 0.58 g; $MgSO_4 \cdot 7H_2O$, 0.50 g; 13% Fe-EDTA solution, 4 mL; Long Ashton trace elements solution, 1 mL; and distilled H_2O, 1000 mL. The solution was adjusted to pH 6.0 with HCl. The trace elements solution was as follows: $MnSO_4 \cdot 4H_2O$, 2.25 mg; $CuSO_4 \cdot 5H_2O$, 0.25 mg; $ZnSO_4 \cdot 7H_2O$, 0.3 mg; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.088 mg; H_3BO_3, 3.0 mg; NaCl, 5.0 mg; H_2O, 1000 mL. The containers were placed in a growth chamber under 6 klx with a photoperiod of 16 h and temperature of 22:16°C (day:night). After 30 days, when the seedlings had entered their exponential growth phase, the sand was flooded with water, and seedlings could be gently lifted out without damaging the fine root systems. Each seedling was placed into a growth pouch (Fortin et al. 1980) containing 15 mL of the following mineral solutions: $(NH_4)_2HPO_4$, 0.13 g; NH_4NO_3, 0.16 g; KCl, 0.45 g; $CaCl_2 \cdot 2H_2O$, 0.44 g; $MgSO_4 \cdot 7H_2O$, 0.37 g; 13% Fe-EDTA solution, 4 mL; Long Ashton trace elements solution, 1 mL; and distilled H_2O, 1000 mL; pH 6.0. Pouches were placed into a growth chamber with the same environmental conditions but 10 klx lighting. One week later, fungi were introduced into the pouches. Five replicates were made for each fungus isolate. Henceforth, the methods described by Godbout and Fortin (1983) were followed for growing the plants and for observation, description, fixing, embedding, and sectioning of roots. At harvest the pH of solutions in the pouches was approximately 5.0. Microscopic observations of ectomycorrhizae were based on longitudinal, radial sections of fixed materials taken from two distinct ectomycorrhizae formed by each fungus species. Macroscopic observations of ectomycorrhizae and macroscopic and microscopic observations of hyphal strands were made directly on fresh material.

Terminology used

The terminology of Zak (1973) is mainly followed in the macroscopic description of ectomycorrhizae and of the extramatrical phase. For the microscopic description of ectomycorrhizae, the following terminology is used. Prosenchyma refers to a tissue forming the mantle in which the hyphal elements are not tightly bound together and where empty spaces exist. Synenchyma is a tissue forming the mantle where the hyphal elements are tightly bound together and no intercellular spaces are present. For greater precision, we divided each of these two main categories into three subdivisions. A tissue was termed shortened when the hyphal elements appeared round to oblong. When the hyphal elements were elongated, the epithet elongated was used. When the section of the tissue possessed as many short as long hyphal elements, the epithet mixed was used. Other qualifiers such as angular and subglobose describe a tissue in which the shape of the cells is angular or subglobose. The term double describes a mantle with an outer, loose, prosenchymatous layer and an inner synenchymatous layer. The term mottled describes a synenchyma in which an unstained layer surrounded the stained content of the hyphal elements and led to a mottled appearance in section. This unstained layer is probably the outer, gelatinized wall of the hyphae and (or) an interhyphal deposit. The term incrusted describes hyphae with surfaces covered by visible incrustations in light microscopy.

For the Hartig net, the terms paraepidermal and periepidermal describe, respectively, a partial and entire hyphal encircling of the epidermal cells (Godbout and Fortin 1983). The epithet uniform describes the constant presence of hyphal penetrations between each epidermal cells. For *Leccinum* species, the term beaded describes unusually large, rounded hyphal elements about 7 – 10 μm in diameter located in the Hartig net; they were best seen in tangential sections. The radial elongation of epidermal cells, as a consequence of fungal infection of the roots, was expressed by a height/width ratio of the cells.

For description of hyphal strands, the terms rim and core were used to describe a differentiated tissue. The rim refers to the outer layer composed of hyphae measuring 2 – 4 μm diam. The core is the inner layer composed of hyphal elements of larger diameter (7 – 8 μm). The different morphological structures of the observed hyphal strands are grouped into seven subdivisions. Type A refers to hyphal strands differentiated into rim and cortex: A1, a broad core surrounded by a rim one hypha thick; A2, a broad core surrounded by a rim two or three parallel hyphae thick; A3, a thin core surrounded by a thick rim of interwoven hyphae. Type B refers to hyphal strands that are not differentiated into a rim and cortex: B1, parallel hyphae; B2, wavy hyphae; B3, interwoven hyphae; B4, parallel, large-diameter hyphae, similar to those of a core.

Results

The root system of aspen developed well under the conditions used. Two weeks were generally sufficient for the roots to completely fill the pouch. Reddening, yellowing, and necrosis of the leaves occurred, but the intensity was randomly expressed among seedlings.

Twenty-nine out of 54 fungus species formed ectomycorrhizae with *Populus tremuloides* seedlings in growth pouches. A minimum of 4 days after the introduction of the inoculum was sufficient for some fungus species to form ectomycorrhizae. Uninoculated controls never became mycorrhizal. A more or less distinctive pattern of morphological and structural characteristics can be outlined for ectomycorrhizae formed by each genus of fungi. These patterns by genus are presented below along with distinctive features for some species.

Ectomycorrhizae formed by species of Hebeloma

The white or pale brown extramatrical phase was composed of an abundant, fleecy mycelium (Fig. 14) with hyphae bearing clamp connections. One hyphal strand of type B1 was observed once with *H. cylindrosporum*. Ectomycorrhizae were white, woolly, and simple. The mantle was very thin (maximum of 20 μm) and was composed of an elongated, compact prosenchyma (Figs. 1*a* and 1*b*). The Hartig net was uniform, paraepidermal to periepidermal, and the epidermal cells were slightly elongated (ratio 1.5:1 to 2.5:1).

The growth of the isolate of *Hebeloma crustuliniforme* was greatly enhanced when placed in pouches next to aspen roots (as compared with its growth in axenic culture).

Ectomycorrhizae formed by species of Cortinarius

The white or pale violet extramatrical phase was composed of a fleecy mycelium and of hyphal strands of type A3 (*C. subporphyropus*) (Fig. 16). Clamp connections were present. Ectomycorrhizae were woolly, simple, and sometimes tortuous, and the structure of their mantles was double but with the inner mantle composed of a compact prosenchyma rather than a synenchyma (Figs. 2*a* and 2*b*). The paraepidermal to periepidermal Hartig net was not uniform, and epidermal cells had a radial elongation ratio up to 2:1.

The isolate CG-47 grew poorly in pure culture, but its growth was greatly enhanced when placed along roots of aspen. This fungus formed sclerotia and some were initiated directly from the mantle of ectomycorrhizae.

Ectomycorrhizae formed by species of Tricholoma

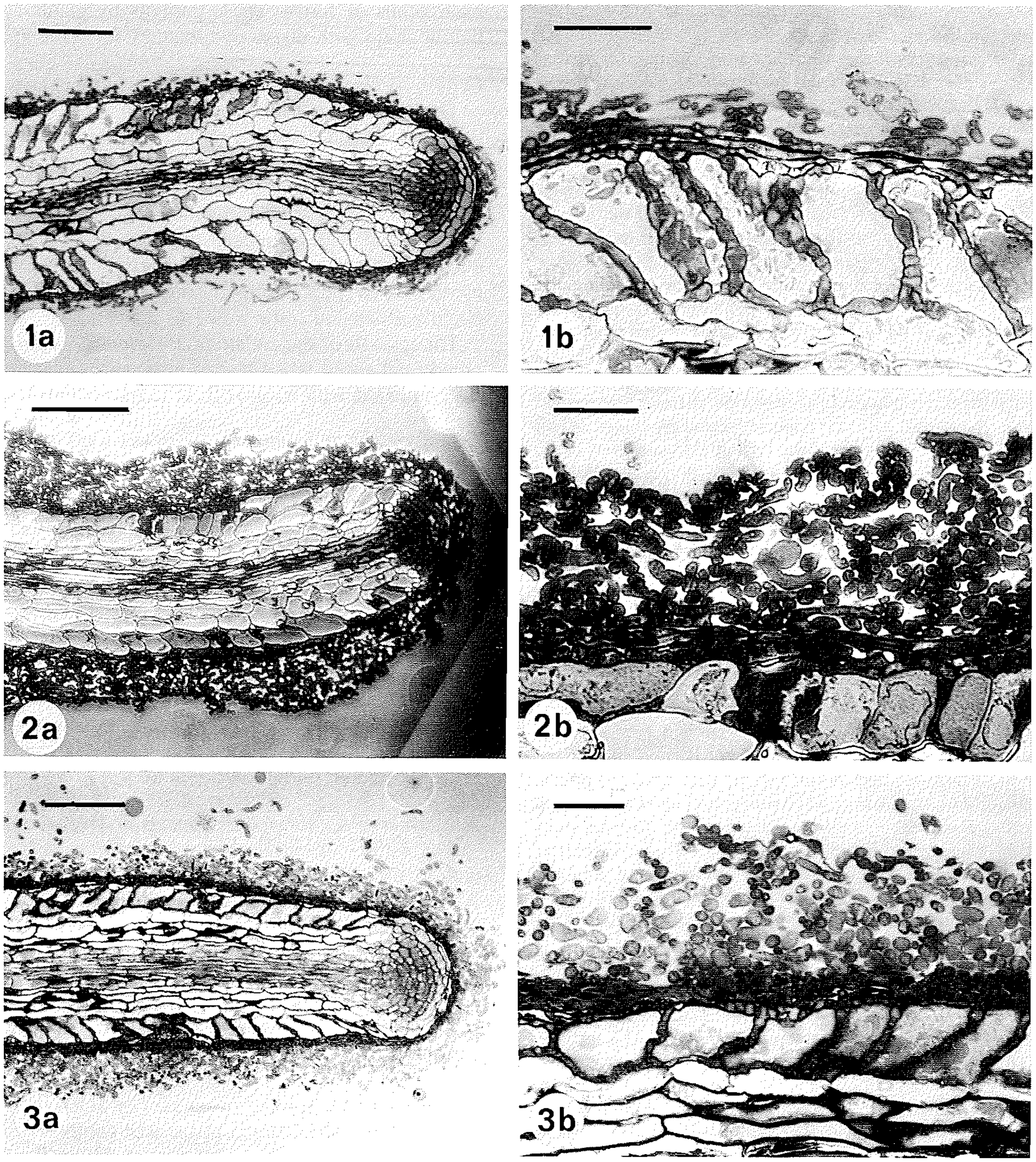
The white extramatrical phase was composed of a fleecy to cottony mycelium (Fig. 15) with smooth hyphal strands of type A2 (as observed with *T. flavobrunneum*). Clamp connections were present in most of the species (absent in *T. cingulatum*). Ectomycorrhizae were white, woolly to cottony, and simple. The mantle was double (not evident in *T. cingulatum*) and elongated to shortened (Figs. 3*a* and 3*b*). The paraepidermal to periepidermal Hartig net was not always uniform and the elongation ratio of epidermal cells was 2:1 to 3.5:1.

Ectomycorrhizae formed by species of Scleroderma

The white extramatrical phase was composed of a weftlike mycelium, and smooth, undifferentiated hyphal strands of type B4 were observed with *S. citrinum*. Clamp connections were present except in *S. areolatum*. Ectomycorrhizae were white at first, woolly to velvety, and branched. The mantle was thick and double with a shortened to mixed structure (Figs. 4*a* and 4*b*). The paraepidermal to periepidermal Hartig net was uniform and the radial elongation ratio of epidermal cells was strong (3:1 to 4:1).

Ectomycorrhizae formed by Pisolithus tinctorius

The golden brown extramatrical phase was composed of a weftlike mycelium, of smooth hyphal strands of type A1 with incrusted hyphae and of sclerotia. Clamp connections were present. The golden brown ectomycorrhizae became dingy cream coloured with age and were coarsely reticulated and ramiform. The thick mantle was shortened, double, with the outer mantle composed of very large hyphae (9 – 12 μm diam.) (Figs. 5*a* and 5*b*). The thick paraepidermal Hartig net was uniform with an elongation ratio of epidermal cells of 2:1.



FIGS. 1–3. Longitudinal radial sections of synthesized ectomycorrhizae of *P. tremuloides* (Figs. 1*a*, 2*a*, and 3*a*) and enlarged portions showing the structure of the mantle (Figs. 1*b*, 2*b*, and 3*b*). Figs. 1*a* and 1*b*. Woolly ectomycorrhiza of *Hebeloma crustuliniforme*. Figs. 2*a* and 2*b*. Woolly ectomycorrhiza of *Cortinarius* cf. *subporphyropus* with its double-structured mantle. Figs. 3*a* and 3*b*. Woolly ectomycorrhiza of *Tricholoma flavobrunneum* with its double-structured mantle. Figs. 1*a*, 2*a*, and 3*a*. Scale = 100 μm. Figs. 1*b*, 2*b*, and 3*b*. Scale = 25 μm.

Ectomycorrhizae formed by Paxillus involutus

The pale brown extramatrical phase was composed of a weftlike mycelium, of smooth hyphal strands of type A1 with incrusted hyphae and of sclerotia. Clamp connections were present. The cream white ectomycorrhizae became deep brown with age and were reticulated and ramiform. The mantle was a mixed prosenchyma (Figs. 7*a* and 7*b*). The paraepidermal Hartig net was uniform and the epidermal cell elongation ratio was about 2.5:1.

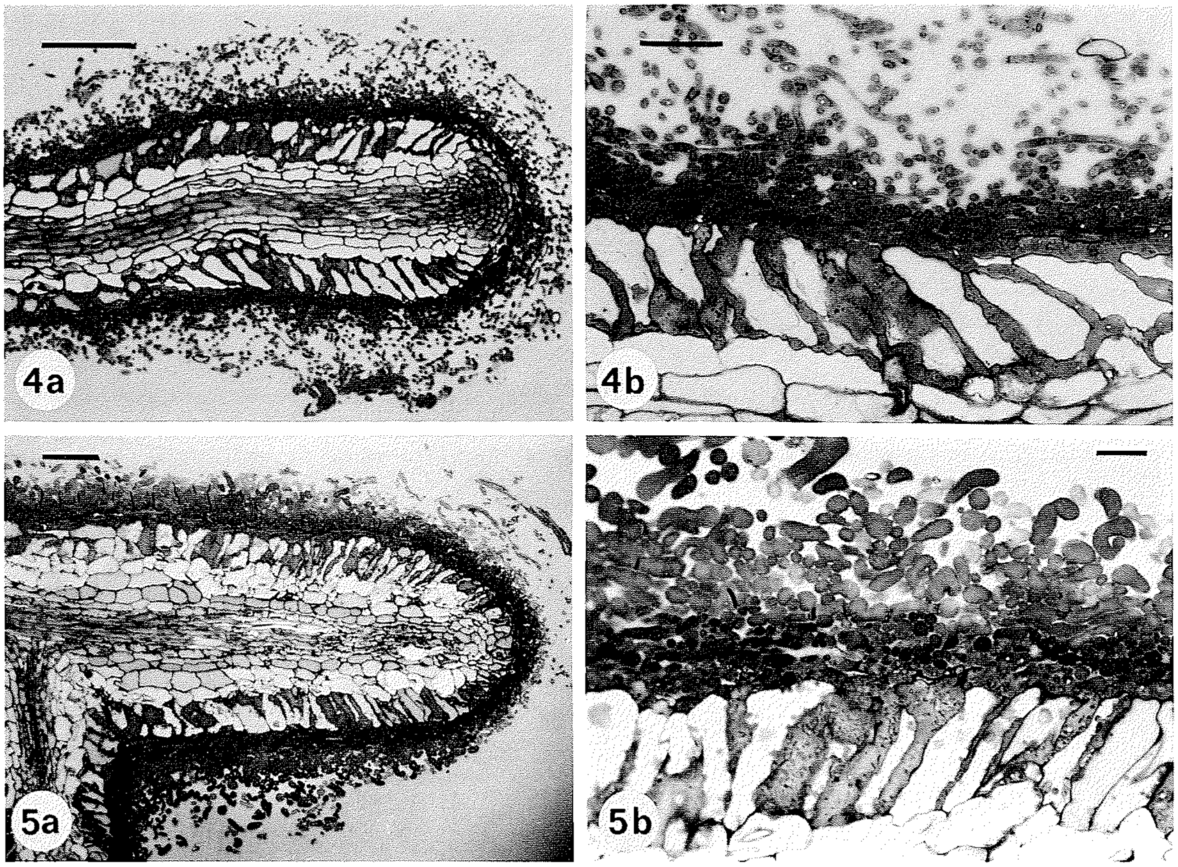
Ectomycorrhizae formed by Laccaria bicolor

The extramatrical phase was composed of hyaline, appressed mycelium with few translucent white, undifferentiated hyphal strands of interwoven hyphae (B3) bearing clamp connections. Ectomycorrhizae were translucent white, simple, and bristly (Fig. 17). The mantle was composed of a mixed synenchyma (Figs. 6*a* and 6*b*). The periepidermal Hartig net was not uniform, and the radial elongation ratio of epidermal cells was about 2:1.

This isolate formed a violet-coloured mycelium in pure culture when young and the violet colour was sometimes observed at the apex of ectomycorrhizae.

Ectomycorrhizae formed by species of Amanita

The extramatrical phase was composed of white, differentiated hyphal strands of type A3 (*A. brunnescens*, *A. mus-*



FIGS. 4–5. Longitudinal radial sections of synthesized ectomycorrhizae of *P. tremuloides* (Figs. 4*a* and 5*a*) and enlarged portions showing the structure of the mantle (Figs. 4*b* and 5*b*). Figs. 4*a* and 4*b*. Woolly ectomycorrhiza of *Scleroderma areolatum* with its double-structured mantle. Figs. 5*a* and 5*b*. Coarsely reticulated ectomycorrhiza of *Pisolithus tinctorius* with its broad outer hyphae of the mantle. Figs. 4*a* and 5*a*. Scale = 100 μm. Figs. 4*b* and 5*b*. Scale = 25 μm.

caria) and sometimes of a poorly developed mycelium. No clamp connections were observed. Ectomycorrhizae were white (at least when young) with a velvety to hoary surface. The mantle was relatively thick and composed of a subglobose to angular, sometimes mottled (*A. muscaria*), shortened synenchyma (Figs. 8*a*, 8*b*, 9*a*, 9*b*). The Hartig net was uniform, paraepidermal to periepidermal, with elongated epidermal cells (ratio 2:1 to 3:1).

The two isolates of *Amanita muscaria* greatly stimulated the formation of root apices (clusters of 10–20 apices). Their very distinctive ectomycorrhizae have a hoary surface (Fig. 18) owing to abundant, approximately 2.5 × 35 μm, cystidiumlike, multiseptate, sometimes branched hyphae (Fig. 9*c*).

Ectomycorrhizae formed by species of Leccinum

The white extramatrical phase ranged from a few hyphae to a poorly developed mycelium with differentiated hyphal strands having a thick rim of parallel hyphae (A3) (as found with *L. aurantiacum, L. griseonigrum,* and L. sp.). No clamp connections were observed. Ectomycorrhizae were white with surfaces mainly reticulate but sometimes densely bristled (*L. griseonigrum*). The mantle was composed of a shortened, mottled synenchyma (Figs. 10*a*, 10*b*, 10*c*). The paraepidermal to periepidermal Hartig net was uniform with an elongation ratio of epidermal cells of 2:1 to 3:1 and contained distinctive beaded hyphae (Fig. 10*c*). Intracellular penetrations of epidermal cells were observed in *L. holopus* and *L. insigne*.

Leccinum griseonigrum greatly stimulated the formation of root apices. Ectomycorrhizae of *L. holopus* became green tinted when injured and their hyphae were incrusted.

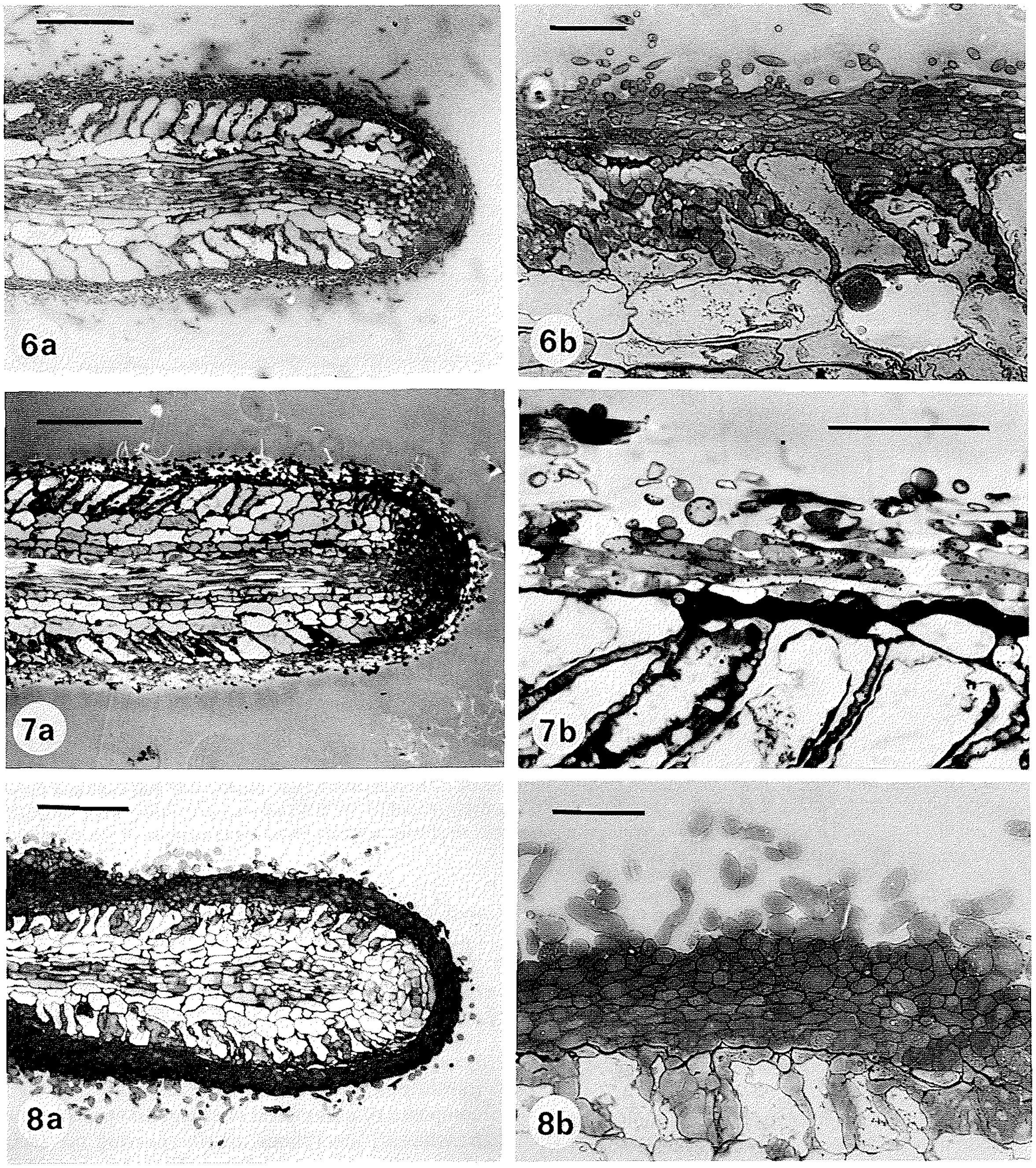
Ectomycorrhizae formed by species of Lactarius

The extramatrical phase was limited to a few hyphae and to undifferentiated hyphal strands of wavy hyphae (B2) (*L. pubescens*). Clamp connections were absent. Ectomycorrhizae were translucent, smooth, and branched (Fig. 19). The mantle was composed of a shortened or elongated synenchyma with a thin, translucent, amorphous matrix covering the surface and enclosing the outermost hyphae (Figs. 11*a* and 11*b*). The paraepidermal to periepidermal Hartig net was uniform, the elongation ratio of epidermal cells was 1:1 to 3:1, and intracellular penetrations were observed in ectomycorrhizae of *L. pubescens*.

Ectomycorrhizae of *Lactarius pubescens* sometimes appeared covered with minute hairs. However, in section, no hyphal elements were found that might explain this surface pattern. In the mantle of *L. hibbardae*, the unusually large hyphae with a poorly stained content might be lacticiferous hyphae (Fig. 11*b*).

Ectomycorrhizae formed by Thelephora terrestris

The extramatrical phase was composed of a translucent, appressed mycelium with hyphae bearing clamp connections. Ectomycorrhizae were slender, hyaline becoming translucent brown, smooth, and simple. The mantle was composed of a thin (10μm), elongated synenchyma (Figs. 12*a* and 12*b*). The



FIGS. 6–8. Longitudinal radial sections of synthesized ectomycorrhizae of *P. tremuloides* (Figs. 6*a*, 7*a*, 8*a*) and enlarged portions showing the structure of the mantle (Figs. 6*b*, 7*b*, 8*b*). Figs. 6*a* and 6*b*. Bristly ectomycorrhiza of *Laccaria bicolor* with its mixed synenchymatous mantle. Figs. 7*a* and 7*b*. Reticulated ectomycorrhiza of *Paxillus involutus* with its mixed, prosenchymatous mantle. Figs. 8*a* and 8*b*. Velvety ectomycorrhiza of *Amanita brunnescens* with its shortened, synenchymatous mantle. Figs. 6*a*, 7*a*, 8*a*. Scale = 100 μm. Figs. 6*b*, 7*b*, 8*b*. Scale = 25 μm.

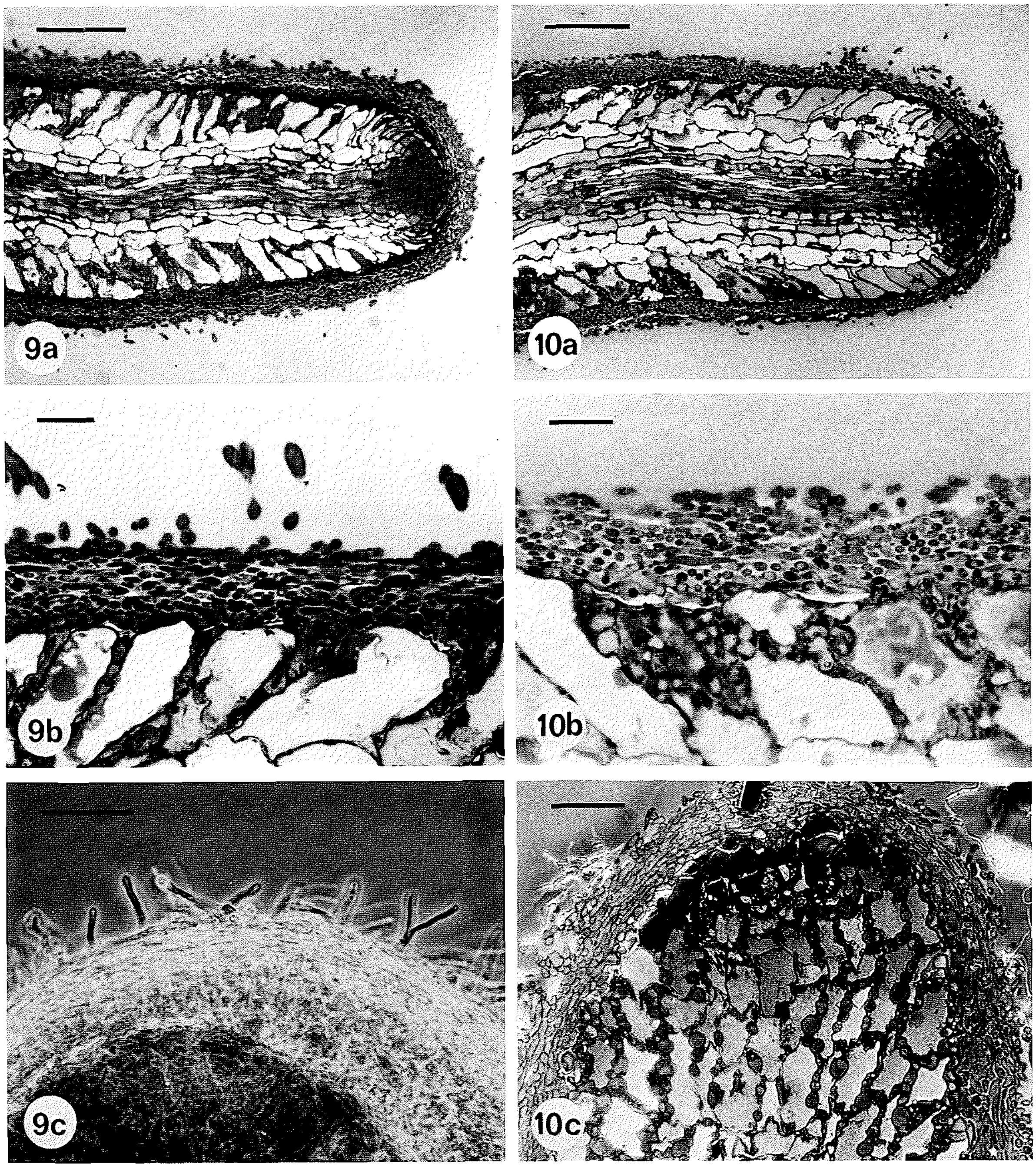
paraepidermal Hartig net was uniform and the epidermal cells elongation ratio was about 1.5:1.

Ectomycorrhizae formed by Cenococcum geophilum

The black extramatrical phase was composed of a loose, weftlike mycelium of thick-walled hyphae lacking clamp connections. Black sclerotia may be observed. Ectomycorrhizae were black, simple, with long hyphae radiating from the smooth mantle surface. Their form was spherical when young, later becoming cylindrical. The mantle structure was a subglobose, shortened synenchyma (Figs. 13*a* and 13*b*). The Hartig net was uniform, periepidermal, with a radial elongation ratio of epidermal cells up to 4:1.

Other fungi

The fungi *Alpova diplophloeus*, *Xerocomus subtomentosus*, and *Rhizopogon* spp. failed to form ectomycorrhizae on aspen seedlings, though they grew to some extent in the pouches (Table 1). Other probable or proven ectomycorrhizal fungi which also failed to form ectomycorrhizae did not grow in the pouches. Some nonmycorrhizal fungi like *Mutinus caninus* and *Dictyophora duplicata* induced a strong discoloration of the



FIGS. 9 and 10. Sections of synthesized ectomycorrhizae of *P. tremuloides*. Figs. 9*a*, 9*b*, 9*c*. Ectomycorrhiza of *Amanita muscaria*. Fig. 9*a*. Longitudinal radial section of its hoary ectomycorrhiza. Fig. 9*b*. Enlarged portion showing the mottled, shortened, synenchymatous structure of the mantle. Fig. 9*c*. Hand section showing the numerous cystidiumlike hyphae covering the surface of the mantle. Figs. 10*a*, 10*b*, 10*c*. Ectomycorrhiza of *Leccinum* spp. Fig. 10*a*. Longitudinal radial section of the reticulate ectomycorrhiza of *Leccinum aurantiacum*. Fig. 10*b*. Enlarged portion of the mantle of *Leccinum aurantiacum* showing its mottled, shortened, synenchymatous structure. Fig. 10*c*. Tangential section through the Hartig net of *Leccinum griseonigrum* ectomycorrhiza showing the beaded hyphae of the Hartig net. Figs. 9*a* and 10*a*. Scale = 100 μm. Figs. 9*b*, 9*c*, 10*b*, 10*c*. Scale = 25 μm.

pouches paper pad.

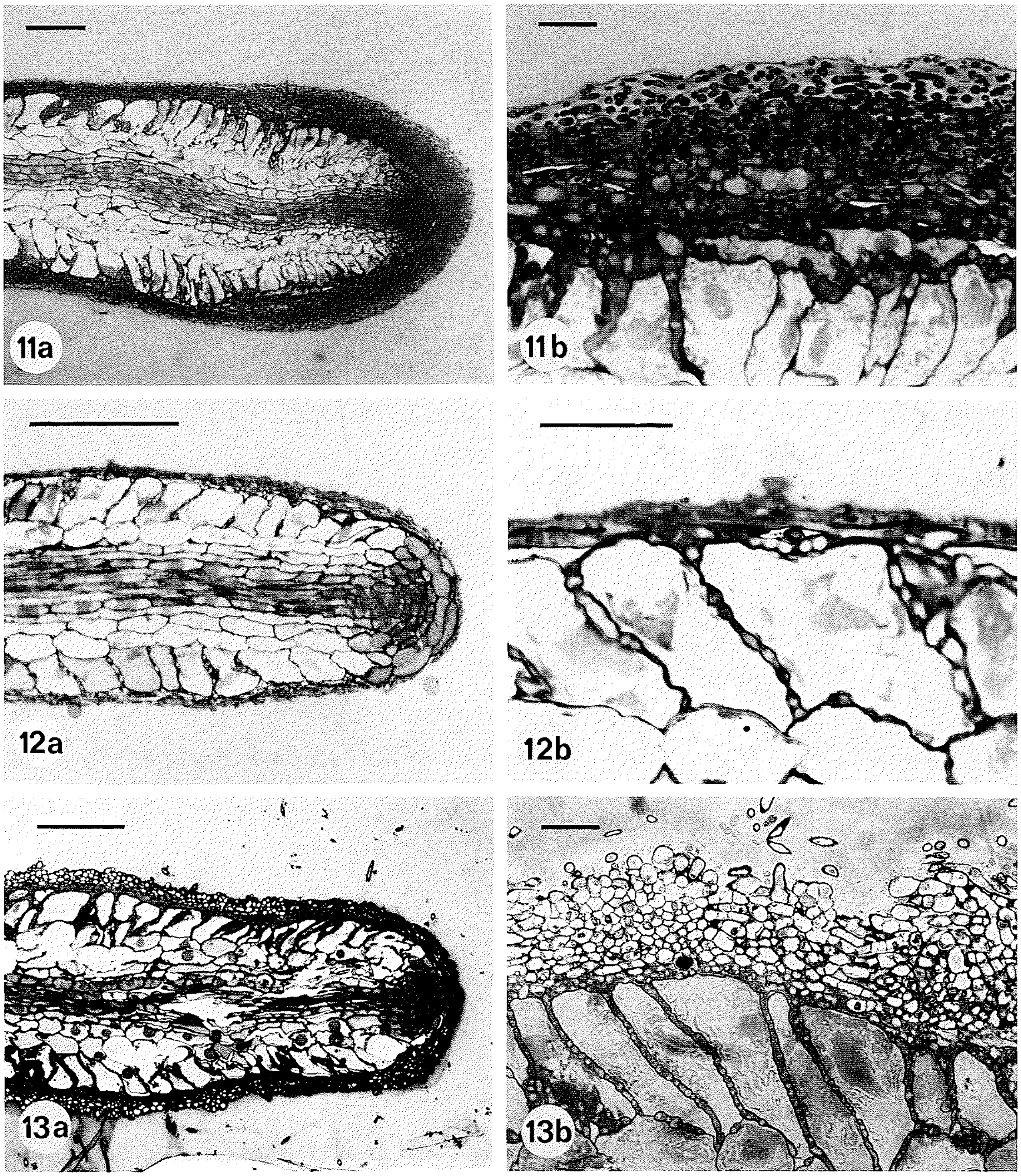
In addition to ectomycorrhizae, five fungus species formed sclerotia in growth pouches and another fungus formed sclerotia without forming ectomycorrhizae. The description of these structures is given below.

Sclerotia formed by Cenococcum geophilum

Three to 12 globular, stiff, black sclerotia, up to 350 μm in diameter, formed in some growth pouches along with ectomycorrhizae. Macroscopically they resembled ectomycorrhizae having a smooth surface and long, emerging, black hyphae. In section the rim (20–30 μm thick) was composed of three to five rows of hyphae 4.5–7.0 μm diam. with the outer rows composed of vacuolated cells. The cortex was formed of hyphal elements 3.0–4.5 μm diam.

Sclerotia formed by Cortinarius *cf.* subporphyropus

Three to 10 sclerotia were observed in each replicate. These stiff sclerotia, about 900 μm in size, were round to ovoid, velvety to woolly, and violet blue fading to cream coloured



FIGS. 11–13. Longitudinal radial section of synthesized ectomycorrhizae of *P. tremuloides* (Figs. 11*a*, 12*a*, 13*a*) and enlarged portions showing the structure of the mantle (Figs. 11*b*, 12*b*, 13*b*). Figs. 11*a* and 11*b*. Smooth ectomycorrhiza of *Lactarius hibbardae* showing the outer amorphous matrix of the mantle and the presence of large hyphal elements which are probably laticifers. Figs. 12*a* and 12*b*. Smooth ectomycorrhiza of *Thelephora terrestris* with its elongated, synenchymatous mantle. Figs. 13*a* and 13*b*. Hairy ectomycorrhiza of *Cenococcum geophilum* with its smooth, subglobose, shortened, synenchymatous mantle. Figs. 11*a*, 12*a*, 13*a*. Scale = 100 μm. Figs. 11*b*, 12*b*, 13*b*. Scale = 25 μm.

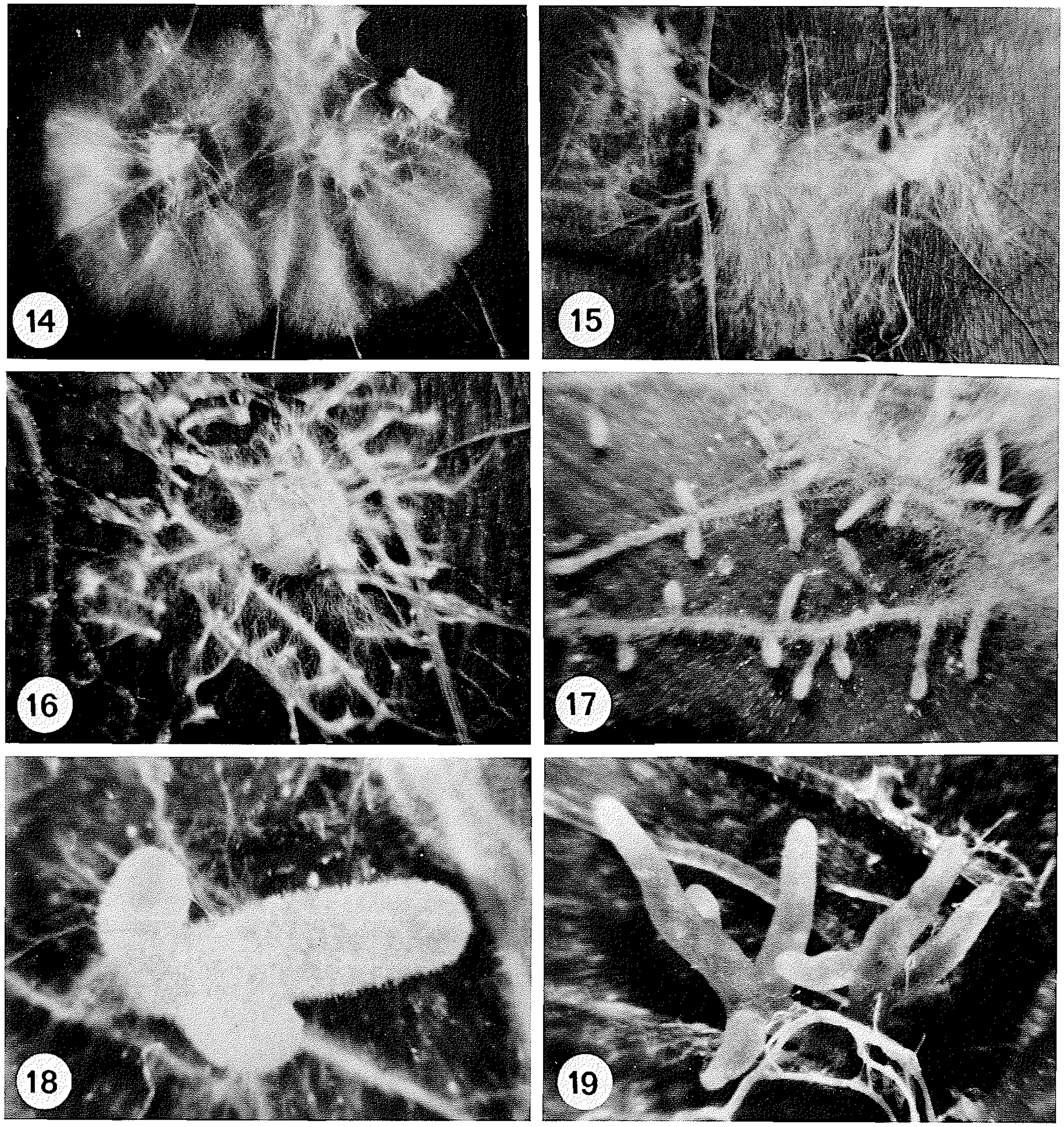
with maturation. The internal structure was not well differentiated. The 35- to 75-μm rim was composed of hyphal elements 6.5–9.0 μm diam. and was looser than the cortex with its 9–14 μm diam. cells.

Sclerotia formed by Paxillus involutus

One to five, globular sclerotia 1 mm in diameter formed in each replicate. Young cream-coloured sclerotia became dark brown, hard, and coarsely interwoven when mature. The rim, 20–25 μm thick, had somewhat vacuolated hyphae 3.5–5.5 μm diam. The cortex had cells that became more vacuolated toward the center.

Sclerotia formed by Pisolithus tinctorius

Two or three spindle-shaped sclerotia reaching 650 × 1200 μm in size were observed in each replicate. Sclerotia were borne on characteristic bright yellow hyphal strands. Young, hoary, chrome yellow sclerotia became reticulated, dark brown, and hard when mature. Two layers, each 25 μm thick with 6–10 μm diam. cells, formed the rim. The outer rim had



FIGS. 14–19. Macroscopic morphology of some synthesized ectomycorrhizae of *P. tremuloides*. Fig. 14. Copious, fleecy mycelium of the extramatrical phase of *Hebeloma crustuliniforme*. 0.65×. Fig. 15. Extramatrical phase of *Tricholoma cingulatum* showing its fleecy mycelium. 0.6×. Fig. 16. Woolly ectomycorrhizae and aerated, fleecy mycelium containing hyphal strands of the extramatrical phase of *Cortinarius* cf. *subporphyropus*. 1.5×. Fig. 17. Bristly ectomycorrhizae of *Laccaria bicolor*. 5×. Fig. 18. Hoary ectomycorrhiza of *Amanita muscaria*. 40×. Fig. 19. Smooth ectomycorrhizae of *Lactarius pubescens*. 25×.

yellow-coloured hyphal elements, while the inner rim possessed hyaline cells. The cortex was made of compact hyphal elements 7–17 μm diam.

Sclerotium-like bodies formed by Laccaria bicolor

Abundant sclerotiumlike bodies (up to 40 in a single pouch) were observed. These loose masses of hyphae were white and bristly and never exceeded 200 μm in diameter.

Sclerotia formed by Gyrodon merulioides

Although this fungus did not form ectomycorrhizae, round to oblong sclerotia averaging 1.5 mm in diameter were sometimes observed. At the beginning they were reticulated and ochre and became smooth, hard, and dark brown when mature. The 35–45 μm thick rim was composed of cells 9–15 μm diam. The cortex was made of two layers. The outer one, 180–360 μm thick, was composed of 9- to 16-μm hyphal elements and the inner one of cells 7–21 μm diam.

Discussion

Data suggest that *Populus tremuloides* forms ectomycorrhizae with a diverse array of fungi. The isolates which kept growing but did not form ectomycorrhizae with aspen in pouches were either nonmycorrhizal (*Dictyophora duplicata*, *Mutinus caninus*, etc.) or were considered specific to other hosts (*Alpova diplophloeus* and *Rhizopogon* spp.). This broad ectomycorrhizal potential seems to be shared by other species of the genus *Populus*. A great diversity of ectomycorrhizae was found on poplars in the field (Dominik 1958; Fontana 1961)

TABLE 1. Fungal isolates, isolation year, associated host, degree of ectomycorrhiza formation, and spread of mycelium of 54 fungus species tested with aspen

Isolate No.	Fungus species	Year of isolation	Associated host[a]	Ectomycorrhiza formation[b]	Spread of mycelium[c] (mm)
DGL 80221E.15	*Cenococcum geophilum* Fr.[d,e]	1980	Pim	+++	5
XX-50	*Cenococcum geophilum* Fr.[e]	1967	Pim	+++	5
CG-205	*Gyromitra esculenta* (Fr.) Fr.	1981	Pim	–	3
CG-202	*Morchella conica* Fr.	1981	Pot, Sa	–	10
CG-281	*Alpova diplophloeus* (Zeller & Dodge) Trappe & Smith[d]	1981	Alc	–	5
CG-245	*Amanita brunnescens* Atk.	1981	Pot, Bep	++	9
CG-259	*Amanita citrina* Schaeff: S. F. Gray	1981	—	++	7
CG-253	*Amanita flavoconia* Atk	1981	—	+	3
CG-40	*Amanita muscaria* (L:Fr.) Pers.: Hook.	1980	Pot	+++	30
CG-59	*Amanita muscaria* (L:Fr.) Pers.: Hook.	1980	Pot	+++	20
CG-237	*Amanita rubescens* (Pers.: Fr.) S. F. Gray	1981	Pid	–	0
CG-246	*Amanita virosa* Secr.	1981	Pis, Bep	–	0
CG-236	*Boletus ornatipes* Pk.[d]	1981	Bep	–	0
CG-16	*Boletus piperatus* Bull.:Fr.	1980	Pot	–	0
CG-43	*Boletus piperatus* Bull.:Fr.	1980	Pot	–	0
CG-235	*Boletus subglabripes* Peck[d]	1981	Bep, Pot	–	2
CG-274	*Cortinarius alboviolaceus* (Pers.:Fr.) Fr.[d]	1981	Pot	+	3
CG-283	*Cortinarius hercynicus* (Pers.) Mos[d]	1981	Alr, Pot	–	0
DM 20.8.80/1	*Cortinarius semisanguineus* (Fr.) Gill.[d,e]	1980	Pim	–	0
CG-47	*Cortinarius* cf. *subporphyropus* Pilàt[d]	1980	Pot	+++	25
CG-275	*Cortinarius* sp.	1981	Pot, Sa	+	5
CG-249	*Dictyophora duplicata* (Bosc) E. Fisher	1981	Pis, Abb	–	25
CG-219	*Gautieria graveolens* Vitt.	1981	Pim	–	0
CG-225	*Gyrodon merulioides* (Schw.) Sing.	1981	Fra	–	25
CG-278	*Hebeloma crustuliniforme* (Bull.: St-Amans) Quél.[d]	1981	Pot	+++	35
75-1	*Hebeloma cylindrosporum* Romagnési[e]	1975	—	+++	20
CG-208	*Hebeloma mesophaeum* (Pers.: Fr.) Quél.[d]	1981	Pot, Sa	+++	20
CG-273	*Hebeloma* cf. *velutipes* Bruchet[d]	1981	Pot	+++	30
CG-211	*Laccaria bicolor* (R. Mre.) Orton	1981	Alr, Pot	+++	25
CG-258	*Lactarius hibbardae* Pk. var. *hibbardae*[d]	1981	Pot	+++	10
CG-277	*Lactarius pubescens* Fr. var. *pubescens*[d]	1981	Bep	+++	25
CG-53	*Leccinum aurantiacum* (Bull.: St-Amans) S. F. Gray[d]	1980	Pot	++	4
CG-22	*Leccinum griseonigrum* Smith, Thiers & Watling[d]	1980	Pot, Pir	+++	4
CG-72	*Leccinum holopus* (Rostk.) Watling	1980	Bep	+	8
CG-52	*Leccinum insigne* Smith, Thiers, & Watling[d]	1980	Pot	+	3
CG-269	*Leccinum subleucophaeum* (Pk.) Sing.[d]	1981	Bep	–	0
CG-33	*Leccinum* sp.	1980	Pot	++	5
CG-82	*Lycoperdon perlatum* Pers.	1980	Pot	–	20
CG-210	*Mutinus caninus* (Pers.) Fr.	1981	Tho	–	15
CG-9	*Paxillus involutus* (Batsch: Fr.) Fr.	1980	Pot	+++	50
76-1	*Pisolithus tinctorius* (Pers.) Coker & Couch[e]	1976	—	+++	30
CG-77	*Psathyrella rigidipes* (Pk.) A. H. Smith[d]	1980	Sa	–	0
CG-261	*Rhizopogon rubescens* (Tul. & Tul.) Tul. & Tul.	1981	Pid, Pir	–	4
CG-218	*Rhizopogon superiorensis* Smith	1981	Pim, Pid	–	4
CG-247	*Rhizopogon* sp.	1981	Pir	–	3
CG-227	*Rhozites caperata* (Fr.) Karst.	1981	Pim	–	0
CG-214	*Russula aeruginea* Lindbl.: Fr.[d]	1981	Pot	–	0
CG-241	*Scleroderma arenicola* Zeller[d]	1981	Acs, Fag	+	4
CG-65	*Scleroderma areolatum* Enrenb.	1981	Acs, Fag	+	4
CG-224	*Scleroderma citrinum* Pers.[d]	1980	Fag	+++	15
CG-240	*Scleroderma citrinum* Pers.[d]	1981	Alc, Bep	++	15
CG-128	*Thelephora terrestris* Ehrl. per Fr.[e]	1980	Pot	+++	35
CG-260	*Tricholoma cingulatum* (Fr.) Jacobasch[d]	1981	Pot, Sa	+++	35
CG-280	*Tricholoma flavobrunneum* (Fr.) Kumm.	1981	Bep, Pot	+++	5
CG-41	*Tricholoma flavovirens* (Pers.: Fr.) Lund.	1980	Pot	–	0
CG-256	*Tricholoma flavovirens* (Pers.: Fr.) Lund.	1981	—	–	0
CG-93	*Tricholoma populinum* Lange[d]	1980	Pot	+++	8
CG-263	*Tricholoma sejunctum* (Sow.: Fr.) Quél.[d]	1981	—	–	0
CG-71	*Xerocomus subtomentosus* (Fr.) Quél.[d]	1980	Pot	–	5
CG-244	*Xerocomus subtomentosus* (Fr.) Quél.[d]	1981	Pim	–	5

[a] Woody plants in the vicinity of sporophore collections. Abbreviations: Abb, *Abies balsamea* (L.) Mill.; Acs, *Acer saccharum* Marsh.; Alc, *Alnus crispa* (Ait.) Pursh; Alr, *Alnus rugosa* (Regel) Fern; Bep, *Betula papyrifera* Marsh.; Fag, *Fagus grandifolia* Ehrh.; Fra, *Fraxinus americana* L.; Pid, *Pinus divaricata* (Ait.) Dumont; Pim, *Picea mariana* (Mill.) B.S.P.; Pir, *Pinus resinosa* Ait.; Pis, *Pinus strobus* L.; Pot, *Populus tremuloides* Michx.; Sa, *Salix* sp.; Tho, *Thuja occidentalis* L.

[b] Degree of ectomycorrhiza formation in growth pouch: +++, >50 mycorrhizal root tips per pouch; ++, between 10 and 50; +, below 10; –, no mycorrhiza formation.

[c] Spread of mycelium in growth pouch with aspen measured from initial inoculum plug to most distant hyphae.

[d] Vouchers at the University of Toronto Herbarium. The isolate CG-260 is placed under the voucher label DGL 81121C.

[e] Fungal isolates provenance: DGL (D. Lahaie) and DM (D. Malloch), Ontario, Canada; XX-50 (C. Richard) and CG-128 (D. Birraux), Québec, Canada; 75-1 (G. Bruchet) and 76-1 (D. Mousain), France.

and heavily ectomycorrhizal root systems were observed during early site colonization (Shuffstall and Medve 1979). Also, carpophores of a wide variety of ectomycorrhizal fungi were found associated with poplars (Peyronel 1917).

All examined sections of aspen ectomycorrhizae showed, without exception, a Hartig net limited to the epidermal layer, a characteristic shared by most of the ectotrophic angiospersms (Godbout and Fortin 1983). Although paraepidermal insertion is usual for epidermal Hartig nets, frequent periepidermal penetrations were also observed in aspen. No clear-cut relationship was found between paraepidermal or periepidermal penetrations and the fungus genus. In periepidermal Hartig nets, hyphae between the epidermis and the cortex of the root were scattered and rarely formed a uniform layer. It is suggested that at least some of the observed paraepidermal Hartig nets would develop into periepidermal ones under different cultural conditions.

A cortical Hartig net has been observed in ectomycorrhizae of other species of poplar (Fontana 1961, 1963; Fontana and Palenzona 1969). However, hyphal penetrations into the cortical region never went further than the first layer of cortical cells. The genus *Populus* seems to share an intermediate position between two groups of angiosperm genera. One including genera such as *Salix, Quercus,* and *Eucalyptus* forms exclusively epidermal Hartig nets (Godbout and Fortin 1983); the other, such as *Cistus* and *Dryas*, forms full cortical Hartig nets (Debaud et al. 1981; Giovannetti and Fontana 1982). A continuum of Hartig nets from the deeply cortical to the superficially epidermal is thus expected to be encountered in angiosperms. The observation of a periepidermal Hartig net in *P. tremuloides* in growth pouches tends to support this conclusion, even though the Hartig net always remained an epidermal one. Passage of sufficient time for hyphae to penetrate the cortex might be an important factor.

The radial elongation of epidermal cells (the so-called palisade layer) seems to occur only when the Hartig net is confined to the epidermal layer. When hyphae penetrated the cortex in poplar ectomycorrhizae, no radial elongation of epidermal cells was observed (Fontana 1961, 1963). Also no elongation of epidermal cells was present in two genera of angiosperms (i.e., *Cistus* and *Drytas*) forming cortical Hartig nets (Debaud et al. 1981; Giovannetti and Fontana 1982). Thus, in epidermal Hartig nets, an increased surface of contact is established between the fungus and the host plant by the elongation of epidermal cells rather than penetrating several cell layers deep in the root.

Some distinct morphological features can be noted for the ectomycorrhizae formed by specific fungi. Those formed by *Amanita muscaria* were easily recognized by their macroscopic appearance owing to the presence of cystidiumlike hyphae. This surface character of the mantle was also observed by Giltrap (1979) and described as "hoarfrost," but no cystidiumlike hyphae were mentioned. Ectomycorrhizae formed by all isolates of *Leccinum* had single hyphae of large diameter in the Hartig net. This structural characteristic was also mentioned by Giltrap (1979) for *Leccinum scabrum* on two species of *Betula* but was probably present in the two other species of *Leccinum* as judged from the wide-ranging diameters of hyphae in their Hartig nets. Beaded hyphae have been reported as a distinctive feature of the pileus cuticule of the genus *Leccinum* as a whole (Smith and Smith 1973). Intracellular penetrations were observed in ectomycorrhizae of two species of *Leccinum* and one species of *Lactarius*. Similar penetrations were also observed in ectomycorrhizae synthesized in pure culture with species of *Leccinum* (Melin 1923) and *Lactarius* (Melin 1923; Giltrap 1979). In the present study, *Leccinum* spp. were very aggressive in their invasion of the epidermal layer, literally destroying this layer of root cells. *Leccinum holopus* also penetrated the cortex of the second-order roots that bear ectomycorrhizae without forming a distinct mantle.

The information presented in this study stresses that ectomycorrhizae formed by congeneric fungi shared similar, distinctive characteristics. A genus level of morphological and structural characterization of ectomycorrhizae has been recognized and applied for *Tuber* (Giovannetti and Fontana 1982), *Lactarius* (Voiry 1981), and *Suillus* (Chumak 1981). Generic characterization of ectomycorrhizae of other genera like *Cortinarius* and *Hebeloma* has been summarized by Voiry (1981) with oaks and beeches and applies in all respects to our results on aspen. An important fact arises from these observations: in structure and morphology the fungal tissue of ectomycorrhizae of a given fungus is independent of the host plant. Several independent reports supported that similar morphologies were formed by a known fungus species on different ectomycorrhizal host plants (Palenzona et al. 1972; Giltrap 1979; Molina and Trappe 1982). The well-known ectomycorrhizae formed by *Cenococcum geophilum* Fr. and *Piloderma croceum* Erikss. & Hjortst are striking examples. Moreover, Zak (1976) and Largent et al. (1980) observed that the macroscopic and microscopic morphologies of external fungal tissues of arbutoid mycorrhizae were similar to those of ectomycorrhizae formed by the same fungal species with conifers. If characterization of ectomycorrhizae is based upon fungal tissues, quantitative rather than qualitative differences are expected to arise from different host plants for a given fungus. However, ectomycorrhizal characteristics, such as the size, the branching pattern, and the location of the Hartig net (epidermal vs. cortical), will vary among host plants as these features are mainly host determined.

In the classification of ectomycorrhizae, it is suggested that the structural types of ectomycorrhizae be defined on the mycorrhizal characters of genera. That is, instead of arbitrarily defined types as in the classification proposed by Dominik (1961), each ectomycorrhizal type would be taxonomically related to an ectomycorrhizal fungus genus. As it also seems possible to omit the host plant as a variable when the fungal tissue only is considered, the task of constructing useful keys would be much more simple than describing each pair of host–fungus combinations, as proposed by Zak (1969).

The present report stresses that microscopic features of ectomycorrhizae are at least as informative as macroscopic ones, if not more so. Thomas and Jackson (1979) pointed out that it was often impossible to distinguish *Picea sitchensis* ectomycorrhizae from macroscopic features alone. Up to now, few reports provided microscopic details of tissue of ectomycorrhizae. Chilvers (1968) emphasized the description of the hyphal strands and of the hyphal pattern from tangential views of the mantle. In this report the structure of the mantle in tangential view was not observed and we feel that it is an information gap.

Descriptions presented here are from synthesized ectomycorrhizae, using the growth-pouch technique. Some of the observed characters may differ from those of natural ectomycorrhizae. Alexander (1981) pointed out that the differences observed in ectomycorrhizae formed by *Lactarius rufus* on *Picea sitchensis* in the field and under aseptic conditions were attributed to ageing processes. The differences concerned mainly the darker colour, the more branched habit, and the collapsing of the outer mantle layers, but the structure of the mantle remained similar.

The presence of sugars in the medium is known to induce artefacts such as a thicker mantle, an isolation of cortical cells, a nontypical extramatrical phase, and a pronounced phenolic response (Giltrap 1979; Thomas and Jackson 1979; Duddridge and Read 1984; C. Godbout and J. A. Fortin, unpublished data).

The growth-pouch method as employed here did not make use of sugars except those contained in the inoculum. However, ectomycorrhizae were in a young state, e.g., not exceeding 25 days old. This technique very likely produces ectomycorrhizae similar to those in the field in respect to their age.

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